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Survey of Cryptosporidia and Giardia Testing Procedures in Michigan

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Division of Infectious Diseases

From December 2000 through February 2001, the Michigan Department of Community Health, Bureau of Epidemiology, noticed an increase (400 percent) of cryptosporidiosis reported by health care providers. The largest increase occurred in two counties. This increase was concerning, as it occurred during the winter months, which historically has seen low levels of cryptosporidiosis. Investigation revealed that the majority of cases were reported from two health care facilities (one in each of the two counties reporting the increase in number of cases) and there were no obvious exposure links between them. Both health care facilities had recently begun to use a rapid immunoassay for the detection of cryptosporidia.

In response, the MDCH Bureau of Epidemiology and the Bureau of Laboratories performed a survey of cryptosporidia and giardia testing procedures in clinical laboratories across the state. The survey was intended to determine the extent to which labs are moving away from microscopic evaluation of these parasitic pathogens and toward the more rapid, less expensive immunoassays. This survey would provide a basis for discussion of the implications of moving toward immunoassays on a broad scale, as clinical labs experience decreased funding and staffing resources dwindle.

The one-page survey was sent out to 110 clinical labs via the broadcast fax system. Respondents were asked whether or not they performed cryptosporidia or giardia testing in-house, or whether samples were sent to a reference laboratory. Labs performing in-house testing were asked if testing for cryptosporidia and giardia was done microscopically (if so, which method) or via immunoassay (if so, which test kit). Labs performing immunoassay, were asked if positive results were confirmed microscopically and if the immunoassay was validated before it was used to report patient results. Eighty-five responses (77 percent response rate) were received and subsequently analyzed. The results are shown in Table 1.

Change in clinical microbiology practice is clearly evidenced by this survey. About 50 percent of microbiology laboratories currently perform any testing for *Giardia lamblia* and/or *Cryptosporidium parvum* on site. Of those who test on site, there is a mix of the standard microscopic procedures and newer EIA techniques, probably dependent on physicians' orders. Less than 10 percent of the labs indicate they confirm positive EIA results for either agent with a microscopic evaluation of the specimen. Given that confirmation is not specifically required, or billable, that figure is not surprising.

There have been several well-publicized incidents of pseudo-outbreaks attributed to past problems with EIA tests, one of which involved detection of cryptosporidia [False-positive laboratory tests for Cryptosporidium involving an enzyme-linked immunosorbent assay-United States November 1997-March 1998. MMWR. 1999.48(01);4-8]. Reagents have been improved but false positives still occur. While not required, it is suggested that confirming cryptosporidia EIA positive results is good microbiology.

In Michigan, *Cryptosporidium parvum* is uncommon and is reportable. This means that false positives, as well as true positives may result in an epidemiological investigation, expending resources unnecessarily. Clinical laboratories dissuaded by the cost of reagents and labor can submit samples to MDCH for confirmation.

The Association of Public Health Laboratories (APHL) has recently taken a position requesting the Food and Drug Administration (FDA) to work with the Centers for Disease

Control and Prevention (CDC), APHL and others to address the impact of direct antigen detection techniques on the ability of public health laboratories to track, confirm, control and prevent communicable diseases. The FDA is being asked to consider the potential impact of the assay on public health programs during the licensure process. Package inserts could carry requirements for confirmatory testing.

Another concern that came to light as a result of this survey is that only two-thirds and one-half (for cryptosporidia and giardia, respectively) of clinical laboratories performed the validation studies required when establishing a new test or switching to a new manufacturer. This is a CLIA 1988 and CAP requirement and applies to all clinical laboratories. Recognizing design and implementation of validation studies can be challenging, MDCH will be exploring ways in which to assist clinical microbiology laboratories in these studies.

What impact will the change to EIA procedures have upon detection of parasites in Michigan? Perhaps a gauge of that impact can be made from an examination of the yield of routine ova and parasite examinations performed at MDCH. The results of 7161 fecal ova and parasite examinations performed from July 1, 1999 through June 30, 2001 are shown in Table 2. Few of these represent samples submitted for confirmation. The majority are routine tests of samples forwarded by laboratories not providing the service. This analysis is limited to some extent as it is unknown how representative this sample is of the population of the state, but it is a gauge of the range of parasites identified in Michigan.

It has been generally accepted that *Giardia lamblia* is the number one parasite in this region of the country. While it is the number one pathogenic parasite, *Endolimax nana* is the most frequently identified parasite at MDCH. Like coliforms in water testing, nonpathogenic parasites are indicator species. While not associated with disease, they do indicate the patient has been exposed to fecal contamination, which may be valuable in patient evaluation. Examination of these specimens by EIA, screening for giardia or cryptosporidia only would have missed 422 pathogenic parasites, about 15 percent of all positives seen at MDCH in this two-year period (some samples had more than one parasite).

This information is provided for clinical microbiology colleagues who may be contemplating or re-evaluating the decision to replace microscopic examinations with EIA exclusively. Comments should be directed to Dr. Patricia Somsel by phone (517-335-8067), or by email (somselp@state.mi.us).

Table 1

Cryptosporidia / _Giardia_ Laboratory Testing Survey Results

	Cryptosporidium	_Giardia_
Does your lab perform in-house testing for _Cryptosporidium_ and/or _Giardia_? YES	**44 (52%)**	**46 (54%)**
The following refers only to labs performing in-house testing		
Does your lab perform microscopic evaluation and, if yes, by which method? YES	**31 (70%)**	**37 (80%)**
Wet Mount	1 (3%)	7 (19%)
Stain Prep	20 (65%)	3 (8%)
Fluorescent Stain	3 (10%)	2 (5%)`
Wet Mount + Stain Prep	1 (3%)	23 (62%)
Stain Prep + Fluorescent Stain	4 (13%)	0 (0%)
Wet Mount + Fluorescent Stain	1 (3%)	0 (0%)
All three methods	1 (3%)	2 (5%)
Does your lab use a concentrated sample for microscopic evaluation? YES	**29 (94%)**	**37 (92%)**
Does your lab perform a rapid immunoassay for _Cryptosporidium_ and/or _Giardia_? YES	**14 (32%)**	**22 (48%)**
Do you confirm positive results obtained by using an immunoassay? YES	**1 (7%)**	**2 (9%)**
Did you validate the immunoassay performance before use? YES	**11 (65%)**	**14 (54%)**

Table 2

MDCH OVA & PARASITE POSITIVES 7/99 THRU 6/01		
	# Pos.	**%Pos.**
Ascaris lumbricoides	59	0.8
Blastocystis hominis*	67	0.9
Chilomastix mesnili	41	0.6
Clonorchis sinensis	1	<0.1
Cryptosporidium parvum	14	0.2
Cyclospora cayetanensis	6	0.1
Dientamoeba fragilis	108	1.5
Diphyllobothrium latum	1	<0.1
Endolimax nana	696	9.7
Entamoeba coli	481	6.7
E. hartmanni	224	3.1
E. histolytica/dispar	145	2
Enterobius vermicularis	6	<0.1
Giardia lamblia	461	6.4
Hookworm	34	0.5
Hymenolepis hartmanni	1	<0.1
H. nana	57	0.8
Iodamoeba butschlii	94	1.3
Schistosoma mansoni	45	<0.1
Strongyloides stercoralis	19	0.3
Taenia	8	<0.1
Trichuris trichiura	62	0.9
*not counted as pathogenic		

Hot Off the Press!

The Fourth Edition of *The Compendium of Methods for the Microbiological Examination of Foods* is now in print. It was edited by the MDCH laboratory director, Frances Pouch Downes, Dr. P.H., and Keith Ito. The text is available from the American Public Health Association (www.apha.org). Downes spent the last three years editing chapters. The fourth edition of the compendium addresses emerging technologies in microbiology and molecular biology. This is particularly significant in this time of the increased attention on food safety and the need for control of foodborne diseases.

Interpretation of STD Testing Results

William S. Sottile, Ph.D. ABMM
Coordinator, Regional Laboratory System

The MDCH Regional Laboratory System has implemented a change in testing procedures for chlamydia or gonorrhea by now using an amplified DNA procedure. DNA amplification is a technique in which a portion of the organism's DNA is copied many times over before attempts are made to detect any specific genetic sequence of the organism of interest. One advantage of this method is that it is now possible to test urine, as well as urethral or cervical swabs, for the genetic material of these organisms. This technology has several important ramifications.

MDCH can now detect chlamydia and gonorrhea at lower concentrations (e.g., very early infections, mild chronic infections) than were detectable before. This should increase ability to identify infected clients, increasing the analytical sensitivity.

After treatment, an organism's DNA may persist at the site of infection for several weeks even though the organism is not viable. Experiments to determine persistence of etiologic DNA of STDs in human subjects would be very difficult to perform. This means that it is inappropriate to use amplified test methods as a test of cure.

The predictive value of a positive result will be lower in a low prevalence population. This holds true for any procedure. In any test method, some percent of the tests will yield a positive test result in the absence of any agent for which the test is designed. The laboratory performs tests which the clinician evaluates in the light of history and all relevant clinical factors. The laboratory does not make a diagnosis; that is the responsibility of the clinician. Laboratory test data is information pertinent to a clinical investigation. Interpretation of the test results must reside with the clinician who must weigh the importance of all the signs, symptoms, risk factors and data together to achieve a working diagnosis. Among the key decisions to perform this test are patient age and history of sexual activity. All factors must be considered again when interpreting test results.

FUN FUNGI.....

Identifying *Aspergillus fumigatus*

Sandy Arduin & Bruce Palma - Mycobacterium/Mycology Unit

Aspergillus fumigatus is the most frequently isolated agent of aspergillosis in humans and is the most commonly found pathogenic *Aspergillus* species. In immunocompromised patients *A. fumigatus* can cause nasal, pulmonary, ocular, cerebral, cardiovascular, bone or organ infection. It is a common airborne mould often associated with compost piles, soil and plant material. The typical colony morphology for *Aspergillus fumigatus* is dark blue-green to grey green turning slate grey with age. *A. fumigatus* is distinguished from other *Aspergillus* species by its columnar heads with uniserate phialides which are found only on the upper half of the vesicle. Conidia are round and can be either smooth or roughened. Conidiophores are colorless to greenish and can be up to 300Fm long. Unlike other pathogenic species of *Aspergillus*, it develops well at 45-50EC.

Aspergillus fumigatus

Last Issue's Picture Quiz Answer:

Photo B is *Ceratocystis* spp., an ascomycete teleomorph of *Sporothrix* spp. The perithecia (fruiting body), found in the teleomorphic state, has a swollen base and a long slender neck. Intact asci are difficult to find because the ascus wall breaks down early in the development of the ascospores. Photo A is the *Sporothrix* anamorph. The picture shows the one-celled conidia grouped in denticles at the tips of the conidiophores. Conidia in rosettes are the prominent characteristic with which the mould phase of *Sporothrix* spp. is identified. This particular isolate did not convert to a yeast phase at 37EC nor did it develop the dematiaceous thick walled conidia commonly associated with *Sporothrix schenckii*. Although environmental isolates of *Sporothrix* spp. have been found to have an ascomycete teleomorph, no teleomorph has been found for isolates of *Sporothrix schenckii* recovered from cases of human infection.

Photo B: Teleomorphic State

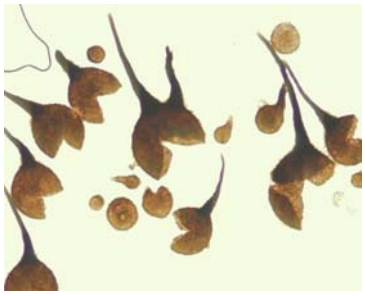
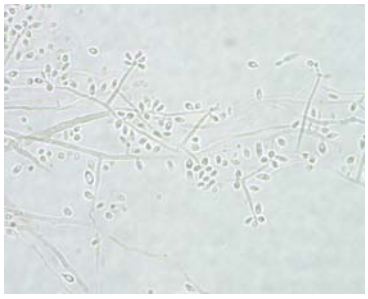
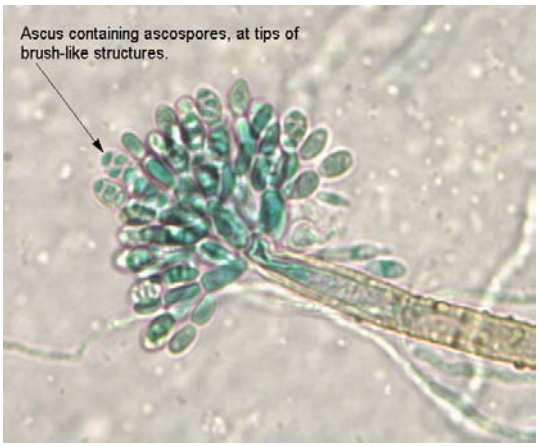


Photo A: Anamorphic State



This issue's picture quiz: What Mould Is This?

This isolate was originally from a blood specimen. The isolate was white, velvety and yeast like. Microscopically it looked like a spindle-shaped yeast. On subculture it developed a dark brown pigment and some aerial hyphae. The picture is of a roughened conidiophore which ended in brush-like structures which contained chains of asci. The asci contained 4-8 bowler hat shaped ascospores. Ascocarps were also present in this isolate.



Small Label Printer Is a Big Help

William S. Sottile Ph.D. ABMM
Lab Director, UP Regional Laboratory

Thanks to Barbara Neaves, R.N. at Marquette County Health Department, for a very helpful suggestion. She suggested using a $25 label printer to label STD swabs and their associated test requests with the patient name and identification number. The labeler is small enough to take into the exam room. Ordinarily the clerk who completes the test request prints out two or more labels as needed. One label is affixed to the test request form in the patient name field and the other label is attached to the specimen. Since the labels are 1) easy to read and 2) true duplicates there are no discrepancies between the label on the clinical specimen and the test request.

Labelers were supplied to 23 of 28 clinics north of Gaylord in a pilot test; they entered service early in January of 2001. The particular model chosen, Brother Ptouch® PT-65, has a feature that allows for two lines of print on a ½" tape. The label is small enough to fit on a culturette® and large enough to be easily read. Figure 1 is a photograph of the Brother Ptouch® PT used in this pilot study and Figure 2 is a photograph of the actual label as it is used in practice.

The U.P. regional laboratory reviews each specimen to ensure that the information on the label is an exact match of the name and identification number on the test request. Whenever the information fails to match, copies of the test request and the specimen label are faxed to the submitting agency for correction and signature. Corrections are faxed back to the U.P. regional laboratory. This activity requires clerical resources of both the laboratory and the submitting clinic. This innovation has decreased this use of resources while assuring reporting quality.

Table 1 contains the results of a quality assurance review of data quality over six months before and after using labeling devices. The percent of errors, based on total specimens for each period, dropped by approximately 75 percent for name and id number errors after the labelers were placed in service. The remaining errors were due to sites not using the labeling device. Other errors (site, date of birth, sex, miscellaneous demographics) were not affected.

Table 1

Period	"Before" July 2000 ~ December 2000	"After" January 2001 ~ June 2001
Total specimens	2,835	2,968
Name errors	52 (1.8%)	13 (0.4%)
ID number errors	23 (0.8%)	5 (0.2%)
Other repairable errors	185 (7.1%)	184 (6.2%)

Figure 1

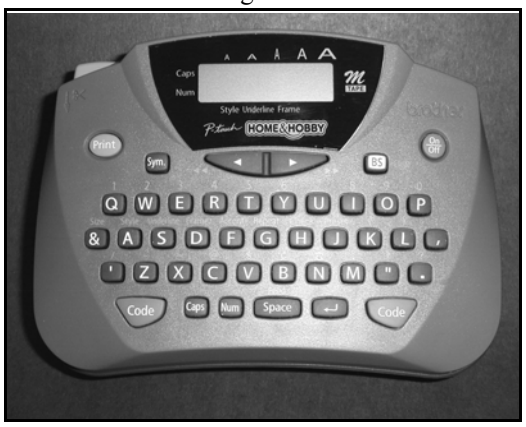
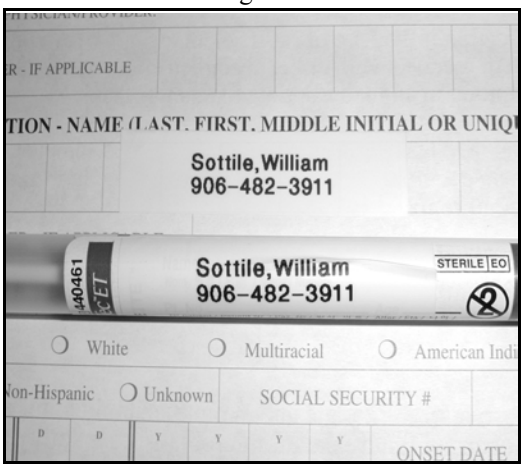


Figure 2



Quirky Bugs...

Robert Jacobson, BS M(ASCP)
Reference Bacteriology Unit

In July 2001, a subdural fluid isolate was received at MDCH for identification. The patient was a 59-year-old male. The initial Gram stain showed a thin curved gram positive rod with some gram negative staining. The colony morphology on sheep blood agar was grey, translucent, circular, slightly raised and entire with weak alpha haemolysis. Growth was better on blood agar than chocolate agar.

In the reference bacteriology unit, unknown isolates are initially inoculated to a short set of biochemicals that include a Triple Sugar Iron (TSI) slant. After 24 hours of incubation, this organism produced an acid reaction on both the slant and the butt (A/A) and exhibited H_2S production. It was also penicillin susceptible and vancomycin resistant with "fuzzy" motility.

One of the few gram positive organisms seen in the clinical laboratory that is H_2S positive is *Erysipelothrix rhusiopathiae*. Subdural fluid would be an unusual source for this organism. Communication with the submitting client revealed that the patient was a heavy alcohol drinker that had fallen at an unknown location. He was admitted and had burr hole procedures. The culture was taken from this area before discharge and the patient did not show up at his post discharge doctor's appointment.

Further biochemical and cellular fatty acid (CFA) analysis confirmed the identity of *Erysipelothrix rhusiopathiae*. *E. rhusiopathiae* infection is a zoonosis. It is carried in the digestive tract of many animals, especially turkeys and swine. The organism is widespread in nature. It is remarkably persistent under many environmental conditions and is parasitic on birds, fish and mammals, especially pigs.

Erysipeloid in humans is a localized cellulitis which develops in two to seven days around an inoculation site. It is contacted through skin abrasion, injury or bite on individuals handling animals or animal products. The lesion is painful and violaceous, hardened with edema and inflammation but no pus formation. It has a clearly delineated border. Adjacent lymphangitis and arthritis may occur. In immunocompromised patients, dissemination and endocarditis does occur with a poor prognosis. Healing can take weeks to months with relapses being common.

Biopsy specimens from lesions are best obtained from deep in the subcutaneous layers at the leading edge of the lesion. Swabs from the surface of the skin are not useful. In disseminated disease normal blood culture techniques are adequate.

Growth occurs on most commonly used laboratory media (e.g., blood agar plates, chocolate agar plates). Colonies may be of two types small and smooth or large and rough, developing after one to three days of incubation. A greening of the agar under the colonies will develop after two days.

Erysipelothrix rhusiopathiae is identified well with Vitek automated systems as well as API Coryne strips. It ferments glucose and lactose and is H_2S positive. The catalase negative organism does not ferment sucrose, xylose or maltose and is negative for nitrate, esculin, urease and gelatin. Supplementation of the liquid media with serum may be necessary. *E. rhusioathiae* will form "pipe cleaner" growth in a gelatin stab incubated at room temperature (22EC).

E. rhusiopathiae is generally susceptible to penicillin, which is the treatment of choice for both the localized and systemic infections. It is usually susceptible to cephalosporins, imipenem, tetracycline, chloramphenicol, erythromycin and the fluoroquinilones and mostly resistant to aminoglycosides, sulfonomides and vancomycin.

This organism is rarely seen at MDCH but with the use of TSI slants, on all isolates, it is quickly suspected when there is production of H_2S. Since most laboratories have this media on hand, it can be used to give presumptive identification when this organism is suspected.

Reference:

Bille J., Rocourt J. and Swaminathan B. 1999. *Listeria, Erysipelothrix* and *Kurthia*. p. 346-356. *In* Murray et al, Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D. C.

Hantavirus Update

Patty Clark, M.P.H.
Viral Serology/Viral Isolation Unit

As of April 16, 2001, 283 cases of Hantavirus Pulmonary Syndrome (HPS) have been identified in 31 states. The distribution of HPS cases includes most of the western half of the United States and some eastern states. Cases of HPS have also been reported in South America, Central America and Canada. Most U.S. cases have been caused by the Sin Nombre virus (SNV). The primary rodent reservoir for SNV is the deer mouse (*P. maniculatus),* whose range includes the continental United States except the eastern seaboard and the Southeast.

Studies indicate the strongest risk factor for HPS infection is increased numbers of rodents in a household. Other risk factors include entering or cleaning rarely opened or seasonal buildings. Occupational risk factors have been recognized but job-acquired SNV infections are infrequent. Travel to and within areas where hantavirus infection has been reported is not considered a risk factor for HPS infection.

The overall case fatality rate for HPS is 38 percent. The fatality rate for cases with an onset since 1/1/94 (after the initial outbreak had receded) continues to fall and is currently 30 percent. Early symptoms include headache, fever, abdominal pain, non-productive cough, nausea and vomiting. Other symptoms may include shortness of breath, dizziness, pain in the large muscle groups and sweats.

There have been no confirmed cases of HPS in Michigan. Wisconsin, Illinois and Indiana have each had confirmed cases.

Hantavirus IgG and IgM testing has been available at MDCH since April 1996. The number of samples tested remains small. Serum samples are tested using an enzyme linked immunoassay for IgG and a capture ELISA for IgM . The acute specimen should be drawn near the time of admission. A second sample should be drawn as late as possible, but no later than 21 days after the acute. Single serum samples are accepted. A sample volume of 2.5 ml of serum is preferred. It will be necessary to submit a further sample for confirmation testing if antibodies are detected initially.

Questions regarding specimen submission or testing should be directed to Patty Clark (517) 335-8102.

Laboratory Response Network Update

James Rudrik, Ph.D.
Bureau of Laboratories

Congratulations to the following laboratories for joining the Michigan Laboratory Response Network for Bioterrorism (LRN) and receiving in-service on the agents most likely to be used in a bioterrorist attack. As of August 31, 2001, 67 laboratories throughout the state have agreed to participate in the LRN and have received training. Any laboratory providing clinical microbiology services may participate in the LRN. MDCH provides on-site in-service education covering the epidemiology, specimen collection and transport, presumptive identification, and disease states produced by *Bacillus anthracis, Yersinia pestis, Brucella* species, *Francisella tularensis, Clostridium botulinum,* and smallpox. Each participating facility also receives a manual describing useful procedures for each of these agents. If your facility has not been contacted to schedule training, contact James Rudrik at (517) 335-8183 for further information or to schedule training.

Allegan General Hospital
Bay Medical Center
Bixby Medical Center
Borgess Medical Center
Bronson Methodist Hospital
Carson City Hospital
Community Health Center Branch County
Community Hospital - Watervilet
Detroit Medical Center
Garden City Hospital
Gerber Memorial Health Services
Hackley Hospital
LakeView Community Hospital
Mecosta County General Hospital
Mercy Hospital - Port Huron
Mt. Clemens General Hospital
Munson Medical Center
Pennock Hospital
Port Huron Hospital
Quest Diagnostics
Sinai-Grace Hospital
South Haven Community Hospital
Sparrow Hospital
Standish Community Hospital
St. John River District Hospital
St. Joseph Mercy Macomb Hospital
St. John Hospital
Sturgis Hospital
VA Medical Center - Ann Arbor
West Michigan Clinical Lab
West Shore Medical Center
Zeeland Community Hospital

Bureau Comings and Goings

The Bureau of Laboratories would like to welcome the following new employees. Virginia Leykem has joined the molecular biology section. Leykem previously worked in the Department of Pediatrics and Human Development at Michigan State University. Barbara Stuart left Oaklawn Hospital in Marshall to take a position as a microbiologist in the microbiology section. Clara Taylor is now the secretary for the division of infectious diseases. Taylor formerly worked for the Department of Consumer and Industry Services in the occupational health laboratory. David Wilkinson comes to the virology section from Lansing's Sparrow Hospital. John Dyke, Ph.D. also left Sparrow Hospital and is currently working is the bureau office. Dyke is working on the National Laboratory System demonstration project.

Kelly Scott of the microbiology section was promoted from laboratory technician to microbiologist in the microbiology section. Bills Crafts has left the microbiology section and is now the unit manager of bacterial and parasitic serology in the virology section. Richard Sheel, Ph.D., of the division of chemistry and toxicology was promoted to the position of clinical health scientist specialist. Sheel recently presented a poster entitled "Adaptation and Modification of Neonatal Screening by Tandem Mass Spectometry Techniques Using Finnigan TSQ Instrument" at the 49th American Society for Mass Spectometry Conference held in Chicago.

Denise Nightingale has left the bureau to pursue her master's at Michigan State University. Barbara Robinson-Dunn, Ph.D., has taken a position at William Beaumont Hospital in Royal Oak.

Congratulations go to Sandy Burch and Cal Frappier. Both have retired from the virology section.

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